UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Michael P. Holsapple, Ph.D.
Health and Environmental Research Laboratory
The Dow Chemical Company
Midland, Michigan 48674

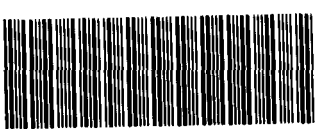
Dear Dr. Holsapple:

Thank you for your letter of September 25, 1997, in which you provided a number of technical comments on toxicology testing guidelines for "Acute Inhalation with Histopathology" and "TSCA Immunotoxicity." I have forwarded a copy of your letter to the Chemical Control Division, which manages the Office of Pollution Prevention and Toxics (OPPT) Chemical Testing Program, for their review and considerationin developing testing actions under Section 4 of TSCA. In addition, I have forwarded the original version of your letter to OPPT's Information Management Division for inclusion in the TSCA Public Docket (Docket Number 0PPTS-42 187-A) that was established for the proposed TSCA Section 4 Test Rule for Hazardous Air Pollutants (HAPs). The proposed TSCA Section 4 HAPs Test Rule was published by the Agency in the Federal Register on June 26, 1996 (61 Fed. Reg. 33 178; FRL-4869-1).

I believe that the above transmittals will ensure that your comments receive appropriate attention. As you know, the Agency cannot incorporate every comment or suggestion received in a rulemaking. If you need further assistance, please feel free to contact me.

Sincerely,

for Susan B. Hazen, Director
Environmental Assistance Division



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The Dow Chemical Company
Midland, Michigan 48674

September 25, 1997

Dr. Susan Hazen
Director, Environmental Assistance Division
Office of Pollution Prevention and Toxics
US EPA, Room E-543B
401 M Street SW
Washington, DC 20460

Dear Dr. Hazen:

Comments on the 870 series toxicology testing guidelines were submitted to EPA by Dow scientists and through the Chemical Manufacturers Association. The guidelines as published in the August 15 CFR do not reflect many of our comments, and we are concerned that these test procedures are inappropriate for HAPS and other EPA related toxicology testing. Since detailed comments were previously sent, only the more important points that were not incorporated in the new HAPS guidelines are noted here. The comments that I would like to address deal exclusively with issues related to immunotoxicity.

Guideline 799.9135. Acute Inhalation with Histopathology.

(1) Pg. 43827; Section iv - "In addition, a phagocytosis assay shall be performed to determine macrophage activity." The incorporation of an imrnune functional test into an acute study is not compatible with other sections of the EPA guidelines. More importantly, the phagocytosis assay using alveolar macrophages has not been well characterized across multiple laboratories. This endpoint cannot be considered to be vaiidated. We do not support the implementation of guideline studies as the appropriate venue to validate an approach, method or test parameter. The incorporation of the macrophage phagocytosis assay will undoubtedly increase the cost of this study and is of limited usefulness after a single exposure.

Guideline 799.9780. TSCA Immunotoxicity.

(1) Pg. 43823; Section H1 - "The guideline now sets the exposure time for the anti-sheep red blood cells (SRBC) assay at 28 days, thus providing information on the effects of the test material on non-specific immunity." The antibody response to SRBC is an indicator of specific immunity - it is incorrect to assume that this test

will provide any information regarding the effects of the test material on non-specific immunity.

(2) There is some confusion in the TSCA guidelines regarding the use of a positive control in the immunotoxicity tests. On Pg. 43823; Section H6, it is stated, "testing laboratories need not perform a positive control after every experiment. Instead, it is sufficient to include this control every six months or whenever new reagents are titrated." On Pg. 43862; Section 3iii, it is stated, "A positive control group with known irnmunosuppressant (e.g., cyclophosphamide) shall be inlcuded in the study." We recommend that the guidelines be amended to reflect the first position - i.e., that it is not necessary to include a positive control with each study. The inclusion of a positive control in every study will increase the cost and will not improve the overall study design as long as the lab regularly confirms the validity and sensitivity of their test methods.

(3) Pg. 43861; Section (2), "In the event the test substance produces a significant suppression of the anti-SRBC response, expression of phenotypic markers for major lymphocyte subpopulations, ... as assessed by flow cytometry, may be performed ..." There are a number of concerns with this recommendation. First, the EPA offers no guidance as to the conditions under which an assessment of phenotypic markers with flow cytometry would be requested in light of clearly positive results with a given test material in the antibody response to SRBC. Second, the NTP database as generated by Mike Luster and co-workers, which is cited as one of the driving forces behind the implementation of immunotoxicity test rules, clearly indicates that immunotoxicity as determined by the antibody response plus thymus weight is just as predictive as immunotoxicity as determined by the antibody response plus phenotypic analysis. Therefore, the more cost-effective option should be allowed as an alternative. Finally, this recommendation is not consistent with the statement on Pg. 43823; Section H1, "EPA incorporated the recommendation of the SAP that the requirement for flow cytometric analysis of lymphocyte ... cell phenotypes be eliminated."

(4) Pg. 43861; Section (2), "If the test substance has no significant effect on the anti-SRBC assay, a functional test for NK cells may be performed to test for a chemical's effect on non-specific immunity." There are a number of concerns with this recommendation. First, the EPA offers no guidance as to the conditions under which an NK functional test would be requested in light of clearly negative results with a given test material in the antibody response to SRBC. Second, the NTP database as generated by Mike Luster and co-workers, which is

cited as one of the driving forces behind the implementation of immunotoxicity test rules, does not provide a single example of a chemical which was judged to positive for immunotoxicity, that was negative in the antibody response but positive in an NK functional test. Therefore, there is no published scientific rationale to support this recommendation. Finally, the EPA has not provided any clarification regarding the concern that an NK functional test has been poorly characterized from the perspective of interlaboratory validation, and the concern that this endpoint is variable.

We believe that quality toxicity testing can be done with greater efficiency and effectiveness if changes are made in the testing guidelines. Thank you for your consideration.

Yours truly,

Michael P. Holsapple

Michael P. Holsapple, Ph.D.

Health and Environmental Research Laboratory